

What is claimed:

1. A method of diagnosing a cellular proliferative disorder of breast tissue in a subject comprising determining the state of methylation of one or more nucleic acids isolated from the subject, wherein the state of methylation of one or more nucleic acids as compared with the state of methylation of one or more nucleic acids from a subject not having the cellular proliferative disorder of breast tissue is indicative of a cellular proliferative disorder of breast tissue in the subject.
2. The method of claim 1, wherein the nucleic acid is selected from Twist, cyclin D2, RAR β 2, WT1, HOXA5, 14.3.3 sigma, estrogen receptor, NES-1, RASSF1A, HIN-1, and combinations thereof.
3. The method of claim 1, wherein the nucleic acid is selected from Twist, cyclin D2, WT1, HOXA5, and combinations thereof.
4. The method of claim 1, wherein the state of methylation of the nucleic acids is determined simultaneously.
5. The method of claim 1, wherein the nucleic acid is selected from RASSF1A, HIN-1, and combinations thereof.
6. The method of claim 2, wherein the state of methylation of the nucleic acid(s) is hypermethylation as compared with the state of methylation of the nucleic acid(s) from a subject not having the disorder of breast tissue.
7. The method of claim 2, wherein the methylation of the nucleic acid is in the regulatory region of the nucleic acid or in the coding region of the nucleic acid.
8. The method of claim 2, wherein the nucleic acid isolated from the subject is obtained from blood, plasma, lymph, duct cells, ductal lavage fluid, nipple aspiration fluid, breast tissue, lymph nodes or bone marrow.

9. The method of claim 6, wherein the duct cells are obtained by a procedure selected from ductal lavage, sentinel node biopsy, fine needle aspirate, routine operative breast endoscopy, nipple aspiration and core biopsy.
10. The method of claim 2, wherein the disorder of the breast is selected from the group consisting of ductal carcinoma *in situ*, lobular carcinoma, colloid carcinoma, tubular carcinoma, medullary carcinoma, metaplastic carcinoma, intraductal carcinoma *in situ*, lobular carcinoma *in situ*, and papillary carcinoma *in situ*.
11. The method of claim 2, wherein determining the state of methylation comprises amplifying the nucleic acid by means of at least one sense primer and at least one antisense primer that distinguishes between methylated and unmethylated nucleic acids.
12. The method of claim 11, wherein the primers hybridize with target polynucleotide sequences selected from SEQ ID NO:1-4, 15-18, 25-36, 41-48, 65-66, 73-76, 81-82, 111-115, 122-123, and combinations thereof.
13. The method of claim 11, wherein the primers are selected from SEQ ID NO:7-14, 21-24, 37-40, 49-64, 69-72, 77-80, 85-90, 116-119, 124-128, and combinations thereof.
14. The method of claim 2, further comprising contacting the nucleic acid with a methylation-sensitive restriction endonuclease.
15. The method of claim 14, wherein the methylation-sensitive restriction endonuclease is selected from the group consisting of MspI, HpaII, BssHII, BstUI and NotI.

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16. A method of determining a predisposition to a cellular proliferative disorder of breast tissue in a subject comprising determining the state of methylation of one or more nucleic acids isolated from the subject,
- wherein the nucleic acid is selected from the group consisting of Twist, cyclin D2, RAR β 2, HOXA5, WT1, 14.3.3 sigma, estrogen receptor, NES-1, RASSF1A, HIN-1 and combinations thereof; and
- wherein the state of methylation of the nucleic acid(s) as compared with the state of methylation of the nucleic acid from a subject not having a predisposition to the cellular proliferative disorder of breast tissue is indicative of a cellular proliferative disorder of breast tissue in the subject.
17. The method of claim 16, wherein the state of methylation of the nucleic acid(s) isolated from the subject is hypermethylation as compared with the state of methylation of the nucleic acid(s) from a subject not having a predisposition to the disorder of breast tissue.
18. The method of claim 16, wherein methylation of the nucleic acid(s) is in the regulatory region of the nucleic acid(s).
19. The method of claim 16 wherein the nucleic acid(s) isolated from the subject is obtained from blood, plasma, breast tissue, lymph, duct cells, ductal lavage fluid, nipple aspiration fluid or bone marrow.
20. The method of claim 19, wherein the duct cells are obtained by a procedure selected from the group consisting of ductal lavage, sentinel node biopsy, fine needle aspirate, routine operative breast endoscopy, nipple aspiration and core biopsy.
21. The method of claim 16, wherein the disorder of the breast is selected from the group consisting of ductal carcinoma *in situ*, lobular carcinoma, colloid carcinoma, tubular carcinoma, medullary carcinoma, metaplastic carcinoma, intraductal carcinoma *in situ*, lobular carcinoma *in situ*, and papillary carcinoma *in situ*.

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22. The method of claim 16, wherein determining the state of methylation comprises amplifying the nucleic acid(s) by means of at least one sense primer and at least one antisense primer that distinguishes between methylated and unmethylated nucleic acid.
23. The method of claim 22, wherein the nucleic acids are amplified simultaneously.
24. The method of claim 22, wherein the primers hybridizes with target polynucleotide sequences selected from SEQ ID NO:1-4, 15-18, 25-36, 41-48, 65-66, 73-76, 81-82, 111-115, 122-123.
25. The method of claim 22, wherein the primers are selected from SEQ ID NO: 7-14, 21-24, 37-40, 49-64, 69-72, 77-80, 85-90, 116-119, 124-128, and combinations thereof.
26. The method of claim 16, further comprising contacting the nucleic acid with a methylation-sensitive restriction endonuclease.
27. The method of claim 26, wherein the methylation-sensitive restriction endonuclease is selected from the group consisting of MspI, HpaII, BssHII, BstUI and NotI.
28. A method for diagnosing a cellular proliferative disorder of breast tissue in a subject comprising:
 - (a) contacting a nucleic acid-containing specimen from the subject with an agent that provides a determination of the methylation state of nucleic acids in the specimen, and
 - (b) identifying the methylation state of at least one region of least one nucleic acid, wherein the methylation state of at least one region of at least one nucleic acid that is different from the methylation state of the same region of the same nucleic acid in a subject not having the cellular proliferative disorder is indicative of a cellular proliferative disorder of breast tissue in the subject.
29. The method of claim 28, wherein the regions of the nucleic acid are contained within CpG-rich regions.

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30. The method of claim 28, wherein the methylation state of at least one region of at least one nucleic acid from the subject comprises hypermethylation when compared to the same region(s) of the nucleic acid in a subject not having the cellular proliferative disorder.
31. The method of claim 30, wherein the nucleic acid is selected from Twist, cyclin D2, RAR β 2, HOXA5, WT1, 14.3.3 sigma, estrogen receptor, NES-1, RASSF1A, HIN-1, and combinations thereof.
32. The method of claim 30, wherein the nucleic acid is selected from Twist, cyclin D2, HOXA5, NES-1 and WT1.
33. The method of claim 30, wherein the nucleic acid is selected from RASSF1A, HIN-1, and combinations thereof.
34. The method of claim 30, wherein the agent is at least one sense primer and at least one antisense primer that hybridize with a target sequence in the nucleic acid.
35. The method of claim 34, wherein the target nucleic acid sequence is selected from SEQ ID NO:1-4, 15-18, 25-36, 41-48, 65-66, 73-76, 81-82 and combinations thereof.
36. The method of claim 34, wherein the primers are selected from the group consisting of SEQ ID NO: 7-14, 21-24, 37-40, 49-64, 69-72, 77-80, 85-90, 114-119, 122-128, 133-134 and combinations thereof.
37. The method of claim 30, wherein the specimen is selected from blood, plasma, breast tissue, biopsy sample, lymph, lymph node, ductal lavage, nipple aspiration fluid and bone marrow.
38. The method of claim 30, wherein the disorder of the breast is selected from ductal carcinoma *in situ*, lobular carcinoma, colloid carcinoma, tubular carcinoma, medullary carcinoma, metaplastic carcinoma, intraductal carcinoma *in situ*, lobular carcinoma *in situ*, and papillary carcinoma *in situ*.

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39. The method of claim 34, wherein the nucleic acid is Twist, cyclinD2, RAR- β , RASSF1A and HIN-1.
40. The method of claim 39, wherein the method employs multiplex methylation-specific PCR.
41. The method of claim 40, wherein the specimen comprises breast duct or ductal fluid.
42. A kit for the detection of a cellular proliferative disorder of breast tissue in a subject comprising
- (a) carrier means compartmentalized to receive a nucleic acid-containing sample from the subject therein;
 - (b) a reagent that modifies unmethylated cytosine nucleotides
 - (c) at least one sense primer and at least one antisense for amplification of CpG-containing nucleic acid, wherein the primers can distinguish between modified methylated and non-methylated nucleic acid.
43. The kit of claim 42 wherein the primers hybridize with a target polynucleotide sequence selected from the group consisting of SEQ ID NO:1-4, 15-18, 25-36, 41-48, 65-66, 73-76, 81-82 and combinations thereof.
44. The kit of claim 42, wherein the primers are selected from the group consisting of SEQ ID NO: 7-14, 21-24, 37-40, 49-64, 69-72, 77-80, 85-90, 114-119, 122-128, 133-134 and combinations thereof.

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